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# Intercropping and environmental seasonality modulate the physiology and growth of *Hancornia speciosa* (Gomes)

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## Abstract

**Background** The recovery process of degraded areas with the implantation of orchards of native species is still little known. Thus, we intend to evaluate the physiological performance of *Hancornia speciosa* during different seasons of the year, cultivated in full sun and in intercropping for recovery of degraded areas.

**Methods** Intercropping with *Syagrus oleracea* (double intercropping); with annual crops (double intercropping); and with *S. oleracea* and annual crops (triple intercropping) were completed over two years at the beginning of each season. Crops (experimental plots) were planted using a Nelder wheel design.

**Results** Over the two years, *H. speciosa* was observed to experience seasonal regulatory changes, some of which were mitigated by the intercropping. The cultivation arrangement influenced the physiology and consequently the growth of *H. speciosa*.

**Conclusions** It is concluded that the double intercropping benefits the growth of *H. speciosa* during the formation of the orchard, and the triple intercropping does not affect growth in relation to full sun. It is recommended the implantation of orchards of *H. speciosa* in recovery areas through intercropping.

## Introduction

Global food production has increased substantially due to the systematization of monocultures. The organization of cultivation practices, mechanization, phytosanitary protocols, and genetic breeding were molded for monocultures in the “Green Revolution” (Pingali 2012). However,

systems intercropped with trees, known as agroforestry systems, can also contribute to improvements in productivity (LER) combined with significant ecological gains (José 2009), obtained by the increase in biodiversity and their respective positive interspecific interactions.

There are several more sustainable cropping modalities than monocultures, as intercropping, which can provide gains in production efficiency of up to 100% (Land Equivalent Ratio—LER) with the same natural resources used in full sun cultivation (Gao et al. 2009). The gains described with intercropping for the Cerrado landscape of Goiás state, Brazil, have varied according to the season, species, and planting layout used (Custódio et al. 2015; Santos et al. 2017a). It is noteworthy that intercropping systems, with different groups of species used, provided results of socio-environmental and economic gains

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(Alves et al. 2015; Gao et al. 2009; Ronald and Charles 2012).

The physiological responses of intercropped crops are extremely important because they are sensitive to adverse conditions for plant growth and productivity and can therefore provide accurate and rapid information about plant vigor (Peng et al. 2009), with the purpose of filtering the desired ones and formatting promising systems.

Despite the thousands of species in the world, few plant species are currently used in food production (Zappi et al. 2015). Aligned with the need for biodiversification of this food source, *Hancornia speciosa* is a species with great potential for use in the food sector given its productive capacity and acceptance by the consumer market (Pereira et al. 2006). It is a widely occurring medium-sized tree species and rustic, native to the Brazilian Cerrado (Vieira Neto et al. 2002). *Hancornia speciosa* is a fruit tree of the family Apocynaceae, a latex producer whose fruit is known as 'mangaba', a Tupi-Guarani word meaning "good thing to eat" (Vieira Neto et al. 2002).

The 'mangaba' fruit has great production potential that is currently underexploited (Ferreira et al. 2018). It is consumed mainly by specific and regional markets either fresh or in the form of juices and ice creams. Several pharmacological uses for *H. speciosa* have also been demonstrated (Marinho et al. 2011; Moraes et al. 2008; Silva et al. 2016).

*Hancornia speciosa* is influenced by environmental conditions such as light, temperature, and water and nutrient availability (Nabout et al. 2016). These environmental factors may directly influence the growth and development of the species and its impact may vary according to the phenological stage of the plant and cropping system (Carvalho et al. 2007).

In this context, the hypothesis that *Hancornia speciosa* has its physiological behavior and growth positively influenced by the cropping system, attenuating the effects of the seasons of the year, enhancing growth increment is fundamental in the establishment of orchards of this species.

Physiological responses that contribute to the growth and development of *H. speciosa* and make it competitive and fit for cultivation have long been sought (Caldas et al. 2009), however, there are no works focused on the hypothesis described.

*H. speciosa* is listed as having either threatened or endangered status. It is an allogamous species that is self-incompatible, with no expression of heterosis or exogamic depression (Collevatti et al. 2016). The maximum sustainable rate of fruit harvest by extractivism for maintaining the native population is 87%. It is noteworthy that the stability of the in situ population is highly dependent on larger adult plants (Lima et al. 2013).

Both extractivism and cultivation of *H. speciosa* are indicated as strategies with the potential for conservation and improvement of the biodiversity and life quality of the communities involved (Bisseleua and Vidal 2008; Caldas et al. 2009; Lima et al. 2013).

Thus, the objective of this study was to characterize the physiological and growth responses of *H. speciosa* Gomes during different seasons of the year when grown in full sun and by intercropping. The experiment aimed to evaluate innovative ways for establishing orchards of *H. speciosa*.

## Materials and methods

### Study site

The experimental site was located in the Cerrado domain (Brazilian savanna) (Batalha 2011), whose climatic conditions are classified as Aw with rainy summer and dry winter (Alvares et al. 2013). The experiment was conducted at the Teaching Farming of Goiano Federal Institute, Iporá Campus (51°09'12" W and 16°25'38" S), at 588 m altitude. The climatic conditions recorded during the test are shown in Fig. 1.

### Soil characteristics

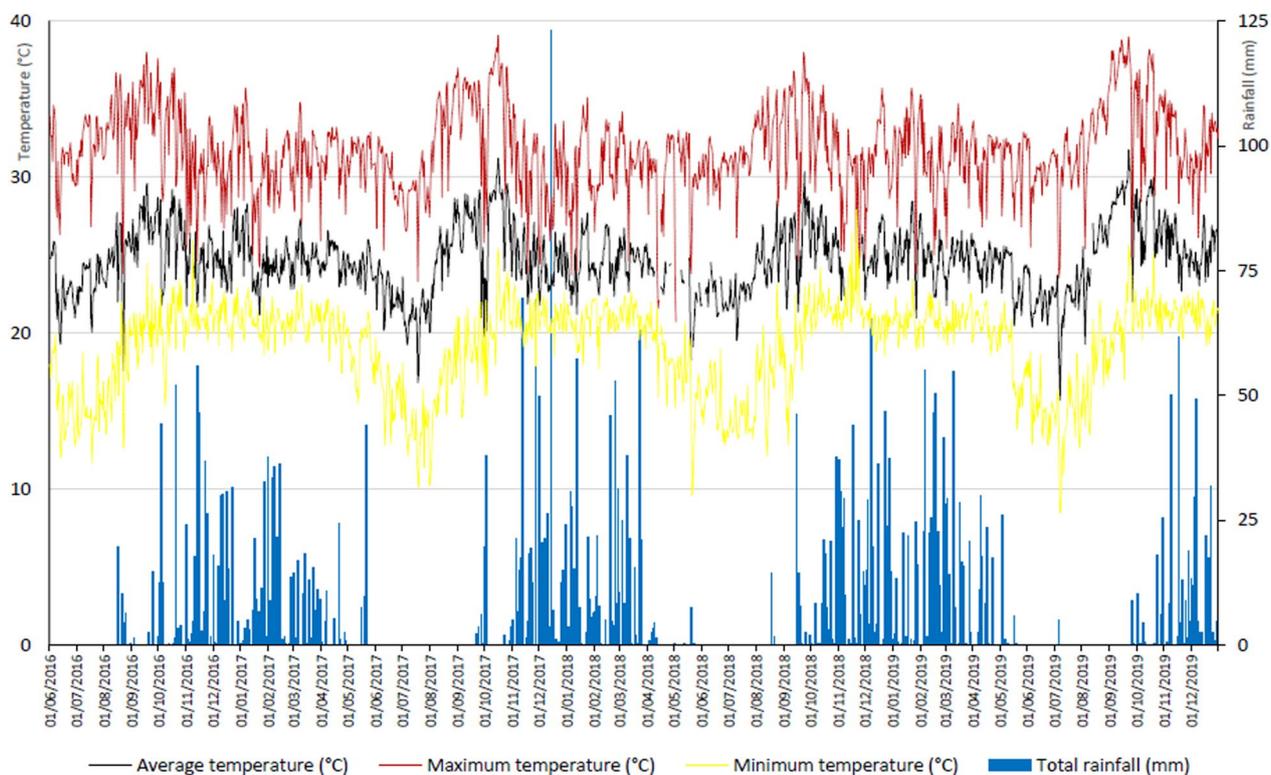
The experiment was conducted in a degraded area, whose soil was classified as Cambisol (Embrapa 2013). The upper soil layer (0.6 m) of the experimental site was removed for use in landscaping, therefore, the experiment was implemented on a subsoil layer, intended for restoration. The physicochemical characteristics of the soil profile at the site before the intervention at the 0.00–0.20 m, 0.21–0.40 m, and 0.61–0.80 m used for implantation of the experiment (Tables 1 and 2).

### Site preparation and planting

The soil was prepared between June 1st and 6th, 2016 with two harrowing operations (28" disks). The first occurred before lime (1.8 Mg ha<sup>-1</sup>) and gypsum (200 kg·ha<sup>-1</sup>) application and the second afterwards, according to the soil correction adapted from Ribeiro et al. (1999).

Seeds of *H. speciosa* were sown in 120 mL tubes in November 2014. The seedlings were later transplanted in February 2015 to 15×15×35 cm (3.78 L) citrus pots filled with Latosol (Embrapa 2013). Seedlings of *S. oleracea* were sown and produced in 3.00 L plastic bags filled with Latosol (Embrapa 2013).

Twenty months after sowing of *H. speciosa* and 8 months after sowing of *S. oleracea*, the seedlings were transplanted to the field. The transplanting beds (pits) measuring 0.15×0.15×0.25 m and 0.40×0.40×0.40 m were dug, and 100 and 500 g of thermophosphate (16% of P<sub>2</sub>O<sub>5</sub>; 12% of P<sub>2</sub>O<sub>5</sub> soluble in citric acid 2%; 16%



**Fig. 1** Maximum, minimum and average temperatures and cumulative precipitation between July 2016 and December 2019. School Farm, Iporá, Goiás, Brazil

**Table 1** Physical–chemical characterization and macronutrient content of the soil in experimental area

Depth (m)	MO g Kg <sup>-1</sup>	Clay	Silt	Sand	pH (ClCa <sub>2</sub> )	Ca cmol <sub>c</sub> dm <sup>-3</sup>	Mg cmol <sub>c</sub> dm <sup>-3</sup>	Al	H	CTC	P* mg dm <sup>-3</sup>	K	Na	S
0.00–0.20	16.0	230.0	130.0	640.0	4.7	2.6	0.6	0.1	4.1	7.75	58	136	3	6
0.21–0.40	7.0	270.0	170.0	560.0	4.2	0.9	0.2	1.5	1.9	4.62	3	48	1	7
0.61–0.80	10.0	500.0	300.0	200.0	4.2	0.5	0.2	1.0	2.8	4.60	2	40	3	4

Iporá, Goiás, Brazil, 2016

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MO, Organic matter; pH, Hydrogen potential; Ca, Calcium; Mg, Magnesium; Al, Aluminum; H, Hydrogen; CTC, Cation exchange capacity; P, Phosphor; K, Potassium; Na, Sodium; S, Sulfur

**Table 2** Characterization of the Ca: Mg ratio, aluminum saturation (M), base saturation (V), saturation by (Ca, Mg and K) and soil micronutrients in the experimental area

Depth (m)	Ca:Mg	----- % -----			----- mg dm <sup>-3</sup> -----						
		M	V	Ca	Mg	K	B	Cu	Fe	Mn	Zn
0.00–0.20	4.3	3.0	46.0	33.8	7.8	4.5	0.2	1.3	27.0	70.0	4.9
0.21–0.40	4.5	55.0	27.0	19.6	4.3	2.7	0.1	1.1	11.0	20.0	0.6
0.61–0.80	2.5	55.0	17.0	10.9	4.3	2.2	0.3	1.1	10.0	10.0	0.3

Iporá, Goiás, Brazil, 2016

B, Boron; Cu, Copper; Fe, Iron; Mn, Manganese; Zn—Zinc

Ca; 6.5% Mg; 6% S; 0.1% B; 0.05% Cu; 0.3% Mn; 9% Si; 0.55% Zn) was applied to each pit for *S. oleracea* and *H. speciosa*, respectively. After transplanting, localized supplementary drip irrigation was employed from July until the beginning of the rainy season in 2016, being equally applied for all the evaluated systems.

Pre-seeding fertilization and topdressing were performed for corn and squash at all cropping. For pre-seeding fertilization, 400 kg ha<sup>-1</sup> of NPK fertilizer (4-30-10) was uniformly applied to the rows (corn) and holes (squash). For topdressing, 200 kg ha<sup>-1</sup> of agricultural urea (45% N) was applied at each crop cycle, divided into two applications (Ribeiro et al. 1999).

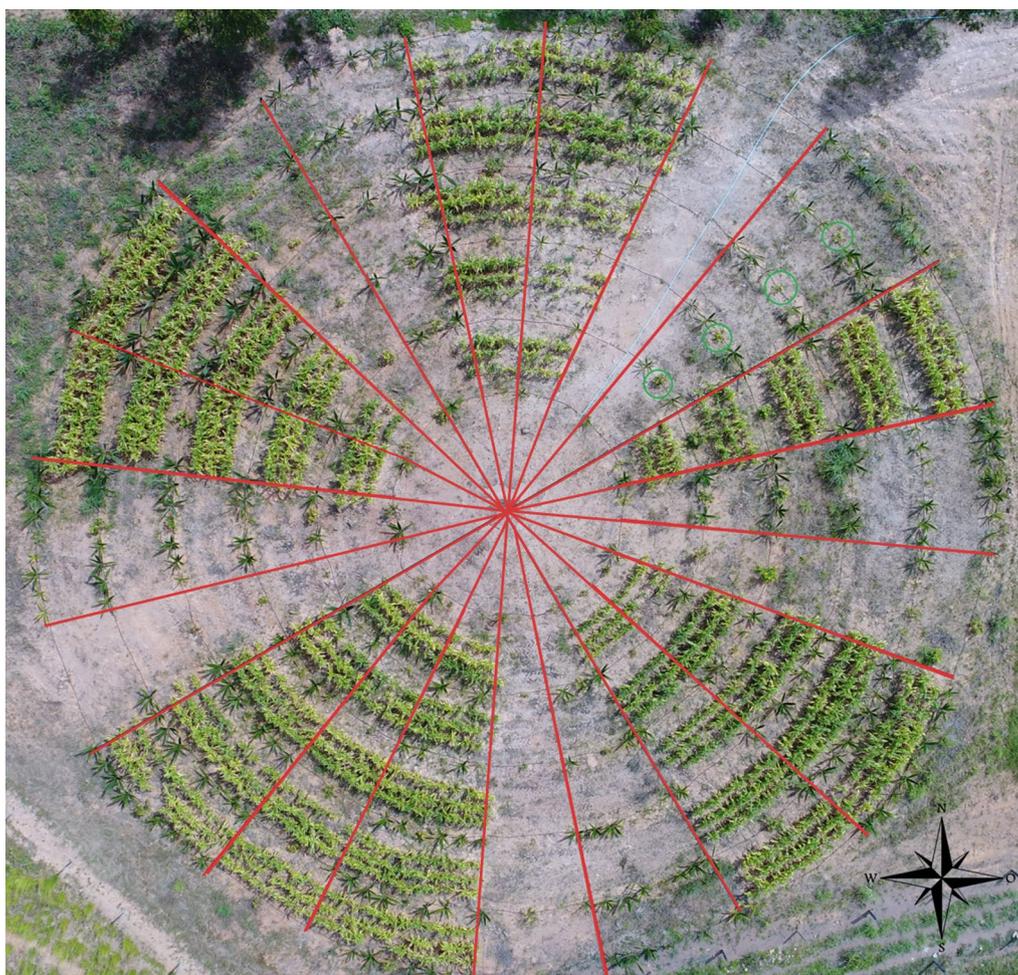
### Experimental design

The distribution of plots and treatments was adapted from the method proposed by Nelder (1962) because each plant would have its effects blocked, allowing the size of the experimental plots to be reduced to one

plant. In this layout, the blocks were placed along the lines formed by the radius in the circumferences, excluding only the inner and outer circumferences (borders) (Fig. 2). Four densities were: 403, 469, 603 e 803 plants ha<sup>-1</sup> de *H. speciosa*, used to block the plots in the direction of the radius.

The experimental design was a complete randomized block design (RBD) with four treatments (*H. speciosa* cropping systems) and 20 replicates. The 20 repetitions were obtained by blocking observations in the five radius and in the four arrangement densities of the four treatments.

One evaluation was performed per season over two consecutive years. A total of 8 ages were evaluated, i.e., the 21st, 24th, 27th, 30th, 33rd, 36th, 39th, and 42nd months after transplanting, performed at the beginning of the seasons (March—Fall, June—Winter, September—Spring, and December—Summer). Therefore, 160



**Fig. 2** View of the experimental area ("Nelder" wheel). March 2018, located 51°09' 12" W and 16°25' 38" S, at 588 m altitude, Iporá, Goiás, Brazil. The red lines indicate the separation between treatments. Green circles are showing the *H. speciosa* plants evaluated

measurements were obtained per treatment for each variable evaluated.

### Experimental treatments

The treatments consisted of four systems of *H. speciosa* ('Mangaba') orchard establishment. The systems were as follows: 1. *Hancornia speciosa* monoculture grown in full sun (**H.s**); 2. *Hancornia speciosa* intercropped with *Syngnathus oleracea* ('Gueroba') cultivated between the plants' rows (**H.s + S.o**); 3. *Hancornia speciosa* intercropped with annual crops (corn and pumpkin, in succession to each harvest sown between rows) (**H.s + Crop**); 4. *Hancornia speciosa* intercropped with annual crops (corn and pumpkin, in succession to each harvest sown between rows) and *S. oleracea* ('Gueroba') grown between the plants in the row (**H.s + S.o + Crop**).

The intercropping systems of *H. speciosa* with annual crops was cultivated by squash (*Cucurbita moschata*) cultivation in all years between September and December evaluations, and corn (*Zea mays*) crop from January to April. Corn was sown in three rows spaced 0.60 m apart, centered in the inter rows of *H. speciosa*. Squash was sown in four holes, located 0.75 m from the *H. speciosa* plants. After emergence, thinning was performed, keeping one *C. moschata* plant per hole, therefore four plants per plot. In the intercropping systems of *H. speciosa* with *S. oleracea*, the two species were simultaneously transplanted to the field at the start of the experiment. *S. oleracea* was arranged in the line (circumference) obeying the spacing of 0.90 m between the plants of *S. oleracea* and also of *H. speciosa* (Fig. 3).

### Cultivation practices

The *H. speciosa* plants did not receive any topdressing. The intercropping systems that included annual crops were cultivated during the dry and rainy seasons with squash (*C. moschata*) and corn (*Z. mays*), respectively. During the dry seasons, squash (*C. moschata*) was cultivated with the aid of local irrigation (drip irrigation). Corn (*Z. mays*) was grown in the rainy seasons, with open pollinated varieties, without irrigation, under a rainfed regime. The pre-planting fertilizers were mixed with the soil removed to open the pits for *S. oleracea* transplanting. Topdressing was applied 0.15 m from the plant stem.

During 90 days of drought in 2016, 2017, 2018 and 2019, all *H. speciosa* plants were equally irrigated, regardless of cropping system. Irrigation in the years of evaluation was carried out between the evaluations of June and September of 2018 and 2019. Period corresponding to the cultivation of pumpkin in the corn inter-harvest, with irrigation depths according to Klosowski et al. (1999).

## Evaluations

### Biometrics of *H. speciosa* plants

For *Hancornia speciosa* growth was evaluated at 21, 24, 27, 30, 33, 36, 39, and 42 months after transplanting, at the same time as the other evaluations. The stem diameter (mm) was determined at 3 cm from the ground using a caliper, and the height (m) was measured using a centimeter ruler.

### Photosynthetically active radiation (PAR)

Photosynthetically active radiation (PAR) was determined using a bar with six PAR sensors model APG-SQ-316 (Apogee, North Logan, UT, USA). The radiation characterization evaluations were performed above and below the *H. speciosa* canopy at 8 am, 12 pm, and 4 pm.

This bar was leveled and positioned 2 cm from the main trunk of *H. speciosa* plants, facing the center of the Nelder wheel, and suspended 10 cm from the ground for the below-canopy readings. For the above-canopy readings, the bar was leveled and positioned 2 cm from the main trunk of *H. speciosa* plants, facing the center of the Nelder wheel, and suspended 10 cm from the top of the plants.

### Gas exchange in *H. speciosa* leaves

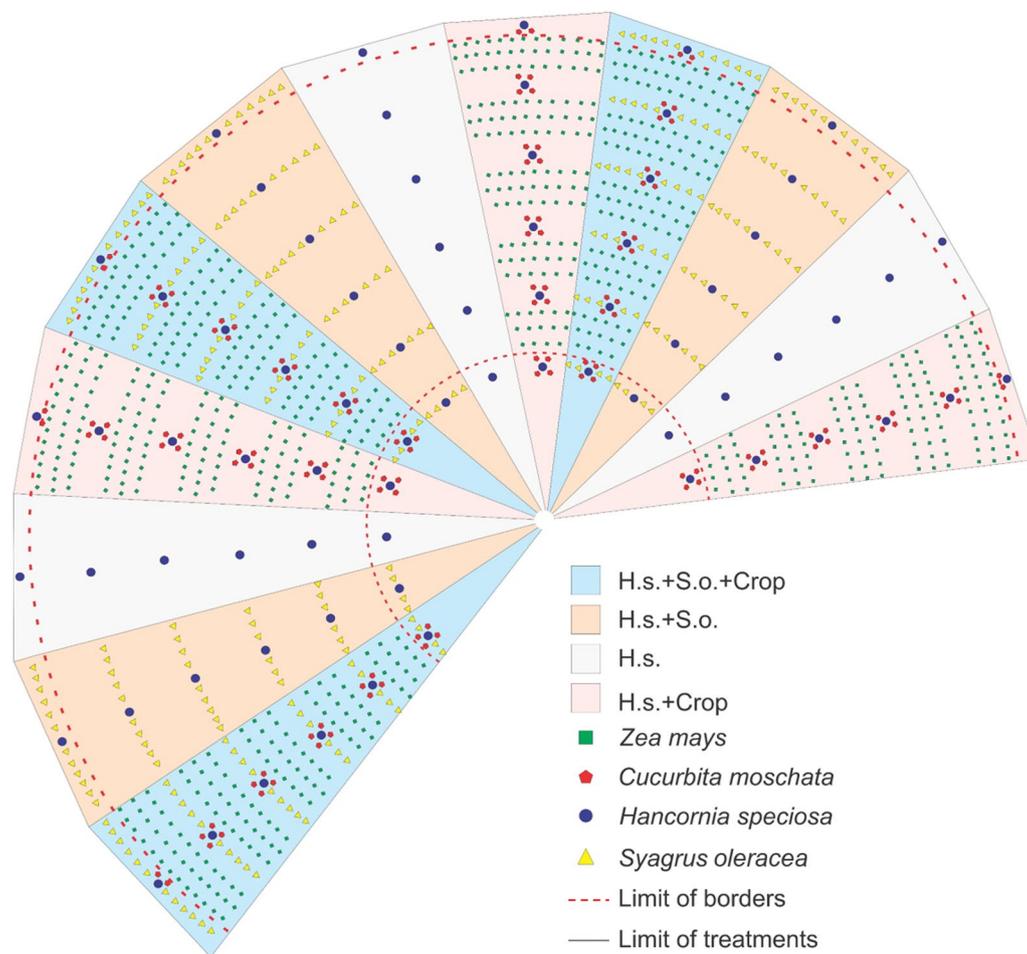
The net carbon assimilation rate ( $A$ ) ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ), transpiration rate ( $E$ ) ( $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ) and stomatal conductance ( $g_{sw}$ ) ( $\text{mol m}^{-2} \text{ s}^{-1}$ ) of *H. speciosa* were measured. These measurements were performed with an infrared gas analyzer (Li-Cor—Li6800 XT, Lincoln, NE, USA). A total of  $1000 \mu\text{mol m}^{-2} \text{ s}^{-1}$  of irradiance was standardized and used at a temperature of  $25^\circ\text{C}$  and  $\text{CO}_2$  pressure of 40 Pa during all evaluations. All measurements were taken from 8:00 to 11:30 am in the third pair of fully expanded leaves located in one of the apex branches.

The water use efficiency (WUE) of *H. speciosa* was calculated by the  $A/E$  ratio ( $\mu\text{mol of CO}_2/\text{mmol of H}_2\text{O}$ ). The instantaneous carboxylation efficiency was calculated by the  $A/C_i$  ratio ( $\mu\text{mol m}^{-2} \text{ s}^{-1}/\mu\text{mol mol}^{-1}$ ) and the  $C_i/C_a$  index ( $\mu\text{mol mol}^{-1}/\mu\text{mol mol}^{-1}$ ) was also calculated.

### Fluorescence of chlorophyll *a* in *H. speciosa* leaves

The test OJIP chlorophyll *a* fluorescence transient was determined with a FluorPen FP 100 portable fluorometer (Photon Systems Instruments; Drasov, Czech Republic). It was evaluated in the third or fourth fully expanded leaf from the apex that was nondetached and had been adapted to the dark for 30 min for complete oxidation of the photosynthetic electron transport system.

The leaves were exposed to a light-saturating pulse ( $3000 \mu\text{mol m}^{-2} \text{ s}^{-1}$ ) with a wavelength of 450 nm for one



**Fig. 3** Experimental design of *H. speciosa* trees and their respective treatments according to the arrangement of crops (squash and corn) and *S. oleracea* palms. Iporá, Goiás, Brazil. **H.s. + S.o. + Crop**—*Hancornia speciosa* intercropped with annual crops (corn and pumpkin, in succession to each harvest sown between rows) and *S. oleracea* ('Gueroba') grown between the plants in the row. **H.s. + S.o.**—*Hancornia speciosa* intercropped with *Syagrus oleracea* ('Gueroba') cultivated between the plants' rows. **H.s.**—*Hancornia speciosa* monoculture grown in full sun. **H.s. + Crop**—*Hancornia speciosa* intercropped with annual crops (corn and pumpkin, in succession to each harvest sown between rows)

second after adaptation to obtain the responses related to the chlorophyll *a* fluorescence transient, according to the JIP test equations.

#### **Analysis of pigments in *H. speciosa* leaves**

Total chlorophyll, flavonoids, anthocyanins, and the nitrogen balance index (NBI) were determined. NBI was calculated as the chlorophyll/flavonoid ratio (Chl/Flav) in the adaxial epidermis of the leaves. A Dualex DX-4 Plus (Force-A, Orsay, France) instantaneous and nondestructive chlorophyll and polyphenol meter was used to record the excitation spectra of chlorophyll fluorescence (Cerovic et al. 2012). For all physiological evaluations, the readings were performed between 8:00 and 11:00 am on the third or fourth fully expanded leaf.

#### **Statistical analysis**

All data were evaluated for the presence of outliers using the *outlierTest* function of the *car* package (Fox and Weisberg 2019) of the R software v.3.4.0 (R CORE TEAM 2020). The identified outliers were removed, and the test was applied again until no outlier was identified. The identified outliers were probably related to extreme results obtained from the measurement equipment, therefore is not related to the experiment sources of variation, then we decide to remove to not compromise the statistical analyses. The normal distribution of the residuals was evaluated using the *shapiro.test* function of R v. 3.4.0. The homogeneity of variances between treatments was determined by the Bartlett test using the *bartlett.test* function in R v. 3.4.0.

All variables were evaluated by mixed models considering the fixed effects of year, season, and system, as well as

the interaction between them. Density of plants, as they present known characteristics equally, formed blocks by density and the blocks were considered fixed effects in the model. Each plant (individual) was considered a random effect. Mixed model analyses were performed with *lmer* function of the *lme4* package (Bates et al. 2015), testing one variable at a time. The *p* value ( $P < 0.05$ ) for the fixed effects was determined by analysis of variance on the mixed model using the sum-of-squares type III method with the function *Anova* (*type = 'III'*) from *car* package in R.

The least squares mean were obtained with the *emmeans* function of the *emmeans* package (Russell and Lenth 2020) and, when one of the fixed effects was significant ( $P < 0.05$ ), the means were compared by Tukey's test adjustment ( $P < 0.05$ ). The graphs of the means and the difference between them were plotted using the *plot* function with the *comparisons = TRUE* command within the *ggplot2* (Wickham 2016) and *emmeans* (Russell and Lenth 2020) packages in R.

We performed Principal Component Analyses (PCA) to identify the relationship between the evaluated variables and how these variables were related to the production systems and seasons evaluated. Principal component analysis (PCA) allowed to summarize the information in our data set, which contained multiple inter-correlated quantitative variables. Multivariate principal component analysis (PCA) was performed using the *PCA* function of the *FactoMineR* package (Le et al. 2008). The graphs of the different PCA dimensions were plotted using the *fviz\_pca\_ind* and *fviz\_pca\_biplot* functions of *factoextra* package (Kassambara and Mundt 2020). The PCA analyses were conducted in groups of variables according to their biological function: Gas Exchange and Biometrics, Pigments and Biometrics, Fluorescence and Biometrics, and Photosynthetically Active Radiation and Biometrics. PCA results were used to perform a hierarchical clustering on the factor map using the *HCPC* function in software R. Table frequencies between the clustering in four groups and cultivation system and season of the year were constructed to evaluate the relation between clusters and each factor.

## Results

The significance levels of the variables evaluated in the experiment for system, season, the system  $\times$  season interaction, and overall mean obtained throughout the observations ( $n \leq 620$ ) are shown in Table 3. Tree density did not affect ( $P < 0.05$ ) any of the variables evaluated, which is probably related to the slow growth of this native species up to 42 months.

The results demonstrate predominantly seasonal effects. Although there are effects of systems and

interactions (systems  $\times$  seasons) on physiological and environmental attributes. Pigments and flavonoids seems to be the variables mainly affected by seasons. Means, standard error and significant difference of the means for production systems  $\times$  season interaction are shown in Additional file 1: Figs. S1–S4.

Percentiles of the variables studied over the 2 years for each treatment, according to the evaluated system, are described in box plots (a visual analysis of the position, dispersion, symmetry, tails, and outliers of the data set). The results bring to light the phytotechnical performance index of *H. speciosa* not described in the scientific literature (Additional file 1: Figs. S5–S10).

Double intercropping (*H. speciosa* + Annual Crops—**H.s + Crop**) and (*H. speciosa* + *S. oleracea*—**H.s + So**) varying above, sometimes below full sun treatment (*H. speciosa*—**H.s**) and triple intercropping (*H. speciosa* + *S. oleracea* + Annual Crops—**H.s + S.o + Crop**) in terms of gas exchange (*A*,  $C_i/C_a$ , and *gsw*) (Fig. 4).

The highest net carbon assimilation rate (*A*) occurred in the H.s + S.o intercropping system compared to full sun system both in the winter and in fall. Great values were observed regardless of the cropping system in the spring (mean of  $11.48 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) which again declined in the summer ( $8.12 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) (Fig. 4).

Greater water use efficiency (WUE) ( $P < 0.10$ ) occurred in the H.s + Crop intercropping system in relation to H.s + S.o + Crop in summer (Fig. 4). It is also noteworthy that the H.s + S.o intercropping system presents greater stability throughout the seasons regarding the instantaneous carboxylation efficiency ( $A/C_i$ ). Therefore, there were no significant changes for this variable in this intercropping system during the fall, winter or spring (Fig. 4).

The transpiration rate (*E*) and the instantaneous carboxylation efficiency change according to the season and do not change according to the cropping system (Fig. 4), as also observed by the multivariate analyses (Fig. 7). The results show no effects of the intercropping systems.

The various parameters related to chlorophyll *a* fluorescence quantum efficiency, quantum yield, and electron transport of PSII were not affected by the *H. speciosa* cropping system (Additional file 1: Figs. S1–S3). Although, season did exert significant effects on these parameters (Additional file 1: Figs. S1–S3).

In the spring, there was a reduction in flavonoids (Flav) and an increase in nitrogen balance (NBI) in the H.s + Crop intercropping system differing from H.s + S.o in NBI and from H.s + S.o + Crop in flavonoids (Fig. 5). It is also noteworthy that the H.s + Crop intercropping system is the only system in which the NBI did not differ between seasons, suggesting greater stability in the face of the environmental variations imposed throughout

**Table 3** Significance of gas exchange, fluorescence, quantum yield, pigment and flavonoids, and photosynthetically active radiation obtained in the cultivation of *H. speciosa*, under conditions of different systems, seasons and systems × seasons interactions

Variable	System	Season	System × Season	Block	Overall mean
<i>Gas exchange</i>					
A	0.351	0.000*	0.040*	0.000*	9.60
E	0.261	0.399	0.637	0.003*	3.96
WUE	0.370	0.063	0.077	0.230	2.89
C <sub>i</sub> /C <sub>a</sub>	0.332	0.006*	0.145	0.212	0.74
g <sub>sw</sub>	0.025*	0.463	0.230	0.041*	0.21
A/C <sub>i</sub>	0.590	0.000*	0.056	0.000*	0.03
<i>Chlorophyll a fluorescence and quantum yield</i>					
F <sub>o</sub>	0.807	0.000*	0.900	0.000*	9460.74
F <sub>v</sub>	0.164	0.000*	0.198	0.000*	34,079.69
F <sub>m</sub>	0.199	0.000*	0.326	0.000*	24,618.95
F <sub>m</sub> /F <sub>o</sub>	0.035*	0.185	0.011*	0.000*	3.67
F <sub>v</sub> /F <sub>o</sub>	0.044*	0.138	0.012*	0.000*	2.67
F <sub>v</sub> /F <sub>m</sub>	0.778	0.001*	0.256	0.000*	0.71
PhiP <sub>o</sub>	0.086	0.171	0.043*	0.000*	0.71
Psi <sub>o</sub>	0.515	0.000*	0.151	0.000*	0.45
PhiE <sub>o</sub>	0.306	0.000*	0.230	0.000*	0.33
PhiD <sub>o</sub>	0.075	0.216	0.042*	0.000*	0.29
PhiPav	0.535	0.000*	0.190	0.111	0.94
PiAbs	0.015*	0.004*	0.015*	0.000*	1.29
ABS/RC	0.730	0.000*	0.702	0.000*	2.60
TR <sub>o</sub> /RC	0.711	0.000*	0.697	0.136	1.65
ET <sub>o</sub> /RC	0.474	0.006*	0.147	0.000*	0.73
Dl <sub>o</sub> /RC	0.511	0.001*	0.123	0.000*	0.83
<i>Pigments and flavonoids</i>					
Chl	0.531	0.000*	0.011*	0.142	19.04
Flav	0.711	0.000*	0.184	0.483	2.08
Anth	0.467	0.000*	0.267	0.096	0.53
NBI	0.652	0.000*	0.019*	0.046*	9.39
<i>Photosynthetically active radiation</i>					
BASE8	0.000*	0.463	0.016*	0.021*	415.54
TOP8	0.000*	0.000*	0.002*	0.000*	677.02
BASE2	0.401	0.191	0.373	0.000*	1252.78
TOP12	0.955	0.000*	0.924	0.000*	2017.76
BASE16	0.000*	0.000*	0.000*	0.000*	481.27
TOP16	0.000*	0.000*	0.229	0.000*	747.09

\* Significant (p ≤ 0.05)

**A**—net carbon assimilation rate (μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>); **E**—transpiration rate (mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>); **WUE**—water use efficiency (μmol CO<sub>2</sub>/mmol H<sub>2</sub>O); **C<sub>i</sub>/C<sub>a</sub>**—internal CO<sub>2</sub> concentration/ambient CO<sub>2</sub> concentration ratio; **g<sub>sw</sub>**—stomatal conductance (mol m<sup>-2</sup> s<sup>-1</sup>); **A/C<sub>i</sub>**—instantaneous carboxylation efficiency (μmol·m<sup>-2</sup> s<sup>-1</sup>/μmol·mol<sup>-1</sup>)

Initial fluorescence (**F<sub>o</sub>** – electrons quantum<sup>-1</sup>), represents all oxidized quinone A; Variable fluorescence (**F<sub>v</sub>** – electrons quantum<sup>-1</sup>) indicates damage to the antenna complex caused by stress; Maximum fluorescence (**F<sub>m</sub>** – electrons quantum<sup>-1</sup>) represents all reduced quinone, the higher the better the energy dissipation in the electron transport chain. The quantum efficiency index of FSII or potential photosynthetic activity (maximum quantum production ratio of competing photochemical and non-photochemical processes in FSII (**F<sub>v</sub>/F<sub>o</sub>** ou kP/KN—electrons quantum<sup>-1</sup>), demonstrates that this parameter is related to the energy captured and the energy dissipated. The index (**F<sub>m</sub>/F<sub>o</sub>**) is the ratio between the maximum and initial fluorescence. PSII Quantum Efficiency, the lower the stress, the greater the stress, indicating damage to the photosynthetic apparatus (**F<sub>v</sub>/F<sub>m</sub>**)

**PhiP<sub>o</sub>** or TR<sub>o</sub>/ABS or φP<sub>o</sub> is the maximum primary photochemical quantum yield index of PSII, non-photochemical dissipation of energy in the form of heat. **PhiE<sub>o</sub>** or ET<sub>o</sub>/ABS or φE<sub>o</sub> or PHI(E<sub>o</sub>) is the index that demonstrates the probability of an exciton moving an electron through the electron transport chain after Quinone A (Q<sub>a</sub>). **Psi<sub>o</sub>** is the probability, in time t = 0, of a trapped exciton moving an electron through the electron transport chain after Q<sub>a</sub>. **PiAbs** or ψABS is the performance index based on absorption, reflects the efficiency of light absorption between capture and transfer of FSII excitation, representing the dissipation of photochemical energy through the formation of ATP and NADPH. **ABS/RC** is the energy absorption per reaction center, the growth in this index indicates an apparent increase in the photosystem's antenna complex to compensate for the high loss of energy as heat. **PhiD<sub>o</sub>** or φD<sub>o</sub> is the energy dissipation quantum yield. **TR<sub>o</sub>/RC** is the flow of energy trapped by reaction center. **ET<sub>o</sub>/RC** is the electron transport flow per reaction center. **Dl<sub>o</sub>/RC** is the specific flow of energy dissipation per reaction center at the level of chlorophylls in the antenna complex, representing disorder at the beginning of the energy transport process. **PhiPav** or φPAV is the time to reach maximum

**Table 3** (continued)

chlorophyll fluorescence (ms)

**ChI**—chlorophyll; **Flav**—flavonoids index; **Anth**—anthocyanins index; **NBI**—nitrogen balance (chlorophyll index/flavonoids index)**BASE8**—Photosynthetically active radiation (PAR) was determine below the *H. speciosa* canopy at 8 am. **TOP8**—Photosynthetically active radiation (PAR) was determine above the *H. speciosa* canopy at 8 am. **BASE12**—Photosynthetically active radiation (PAR) was determine below the *H. speciosa* canopy at 12 pm. **TOP12**—Photosynthetically active radiation (PAR) was determine above the *H. speciosa* canopy at 12 pm. **BASE16**—Photosynthetically active radiation (PAR) was determine below the *H. speciosa* canopy at 4 pm. **TOP16**—Photosynthetically active radiation (PAR) was determine above the *H. speciosa* canopy at 4 pm

the year. The other systems did fluctuate according to the season.

Different seasons had different effects on each evaluated variable, for example the reduction in chlorophyll *a* in the spring, the increase in flavonoids in fall, the reduction in anthocyanins in fall, and the reduction in nitrogen balance in the spring (Fig. 5).

The cropping system showed effects on stem diameter and height starting on 36th and 39th months after transplanting *H. speciosa* plants, respectively (Fig. 6). It is worth noting the need for long periods of evaluation to detect effects on Cerrado trees.

The fitted linear regression models for the different systems confirmed the H.s+Crop and H.s+S.o (double intercropping systems) as suitable for the height growth of *H. speciosa*. In full sun (H.s) and triple intercropping (H.s+S.o+Crop) performed similarly throughout the evaluated period. It is worth noting that triple intercropping did not affect *H. speciosa* growth when compared to the reference (Full sun).

The angular coefficients of the models show that triple intercropping (H.s+S.o+Crop) tended to increase the height at the expense of a smaller stem diameter. In the case of in full sun (H.s), there was an inverse behavior.

These results were reinforced by the photosynthetically active radiation (PAR) (Additional file 1: Fig. S4), which reached from the top to the bottom of *H. speciosa* plants. The effects obtained corroborate expected trends. The PAR values were higher in the Hs system and lower in the H.s+S.o+Crop system. In full sun and triple intercropping are exposed to higher and lower light incidence, respectively (Additional file 1: Fig. S4).

Although different physiological responses occurred throughout the seasons, thanks largely to the meteorological conditions imposed on the plants during growth, the double intercropping systems mitigated the effects of seasons, leading to greater stability. On the other hand, the triple intercropping promotes higher competition between plants and species opposing with benefits of intercropping.

The PCA performed with Gas exchange+Biometrics (Fig. 7), Pigments+Biometrics (Fig. 8), Fluorescence+Biometrics (Fig. 9) and Photosynthetic active radiation+Biometrics (Fig. 10) data were robust and clearly identified which variables were positively or negatively correlated with the evaluated seasons and systems.

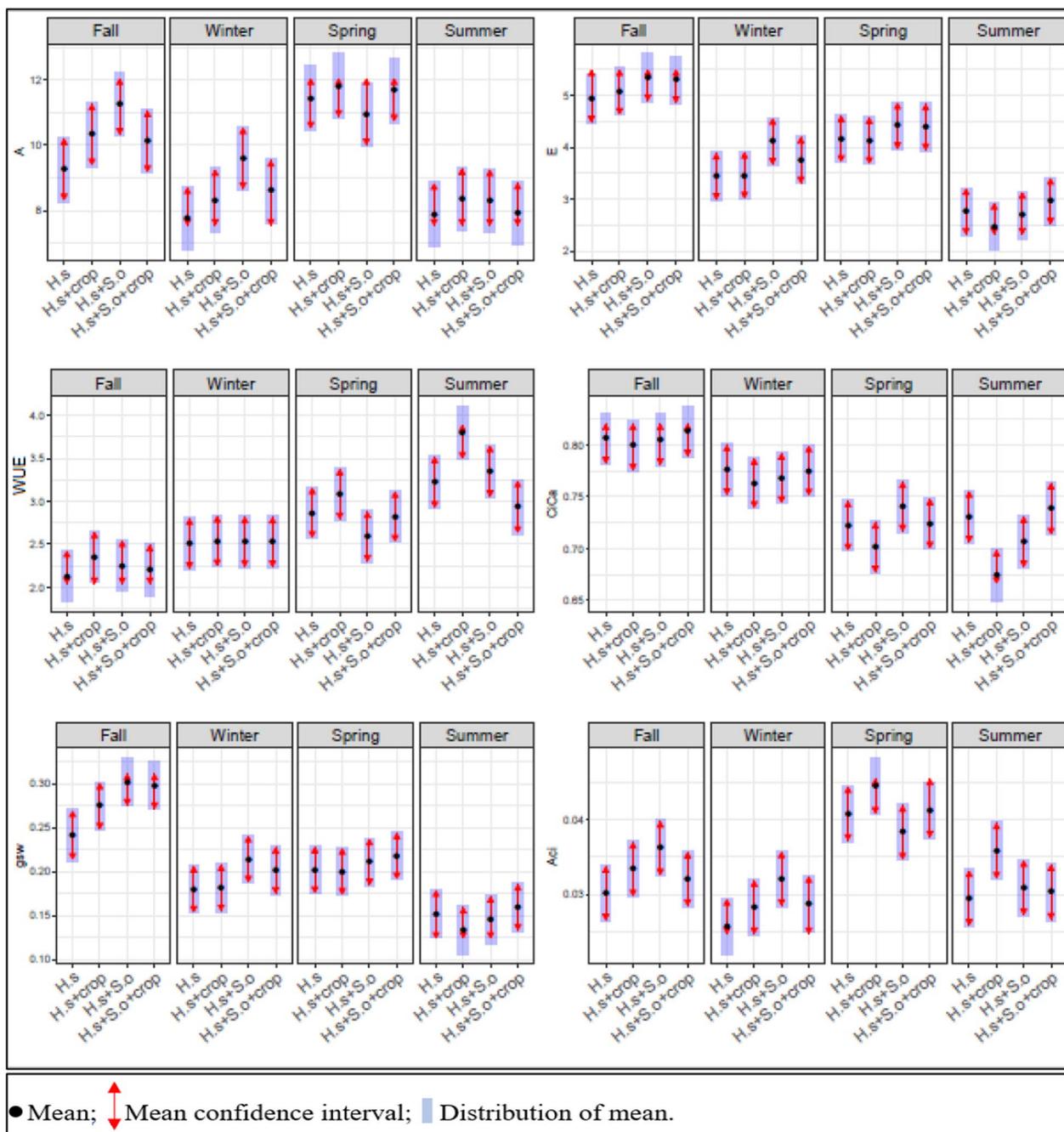
The PCA with physiological and environmental parameters showed some separation of the seasons and no separation between the systems (Figs. 7, 8, 9, 10). The PCA with photosynthetically active radiation showed a clear separation of winter in one of the clusters and no separation for systems. PCA clusters for gas exchange did not show any clear separation between systems and seasons. Clusters with PCA of fluorescence variables showed that none Spring observations in cluster 4 and the other seasons were equally represented, what showed some different behavior of spring in relation to other seasons. Same behavior was seen for summer observations of the clustering with pigments variables, showing a different pattern in pigments measured at summer in relation to other seasons. According to the PCA, the lowest net carbon assimilation rates corresponded to full sun (Hs) and the highest to double intercropping (H.s+S.o). WUE was highest for H.s+Crop and lowest for triple intercropping (H.s+S.o+Crop).

Spring is the season in which PAR was most different from the other seasons, followed by winter. The summer and fall have similar PAR and had intermediate effects compared to the other seasons according to the univariate analyses. Spring had higher anthocyanin and lower Flavonoids content than Fall and Summer seems to be related to higher NBI and Chlorophyll *a* than Spring (Fig. 9).

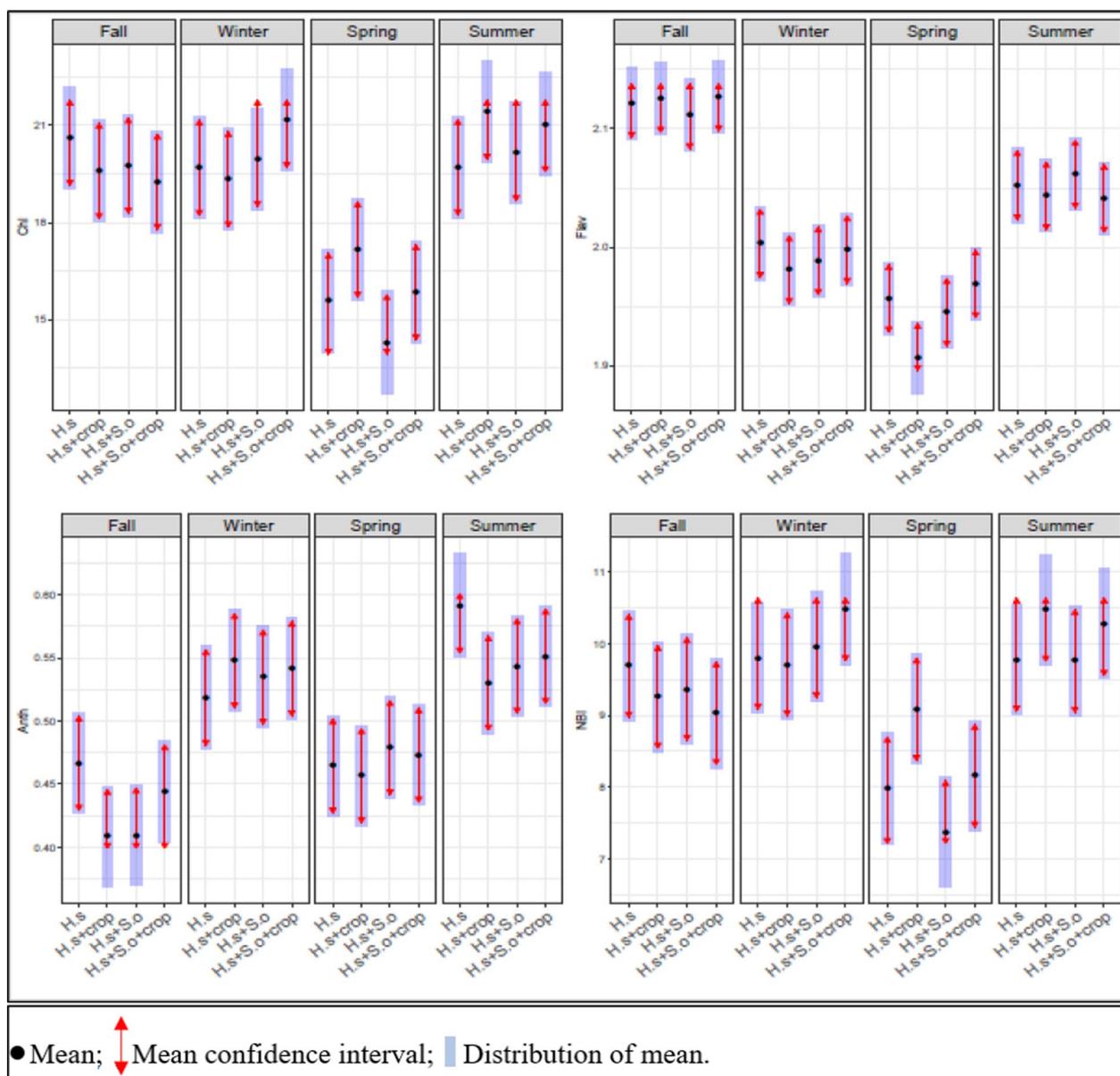
Results of fluorescence variables did not differ between systems, but were able to significantly separate Spring from Fall (Fig. 9). Spring mainly had higher TRoRC, Fo and ABSRC compared to Fall and Winter. Figure 10 shows clear separation of Winter in relation to other seasons. BASE12, BASE 16, TOP 16 and TOP 12 were lower in Winter and higher in the other seasons.

## Discussion

In intercropping systems, all components of the system must be as complementary as possible to ensure favorable environmental conditions for the expression of productive potential (Barbosa et al. 2019). However, the slow growth of the species investigated explains the absence of the population density effect. According to Lima et al. (2013) and Pereira et al. (2006), effects are expected only in the productive phase, when *H. speciosa* is fully developed.



**Fig. 4** Effects of system and season on gas exchange parameters ( $P < 0.05$ ). The blue bars are the confidence intervals of the means, and the red arrows are the comparisons between them. If an arrow from one mean overlaps the arrow of another treatment, there are no differences between means ( $P > 0.05$ ) by Tukey's test. **A**—net carbon assimilation rate ( $\mu\text{mol CO}_2\text{m}^{-2}\text{s}^{-1}$ ); **E**—transpiration rate ( $\text{mmol H}_2\text{O}\text{m}^{-2}\text{s}^{-1}$ ); **WUE**—water use efficiency ( $\mu\text{mol CO}_2/\text{mmol H}_2\text{O}$ );  **$C_i/C_a$** —internal  $\text{CO}_2$  concentration/ambient  $\text{CO}_2$  concentration ratio; **gsw**—stomatal conductance ( $\text{mol m}^{-2}\text{s}^{-1}$ );  **$A/C_i$** —instantaneous carboxylation efficiency ( $\mu\text{mol m}^{-2}\text{s}^{-1}/\mu\text{mol mol}^{-1}$ ). **H.s. + S.o. + Crop**—*Hancornia speciosa* intercropped with annual crops (corn and pumpkin, in succession to each harvest sown between rows) and *S. oleracea* ('Gueroba') grown between the plants in the row. **H.s. + S.o.**—*Hancornia speciosa* intercropped with *Syagrus oleracea* ('Gueroba') cultivated between the plants' rows. **H.s.**—*Hancornia speciosa* monoculture grown in full sun. **H.s. + Crop**—*Hancornia speciosa* intercropped with annual crops (corn and pumpkin, in succession to each harvest sown between rows)

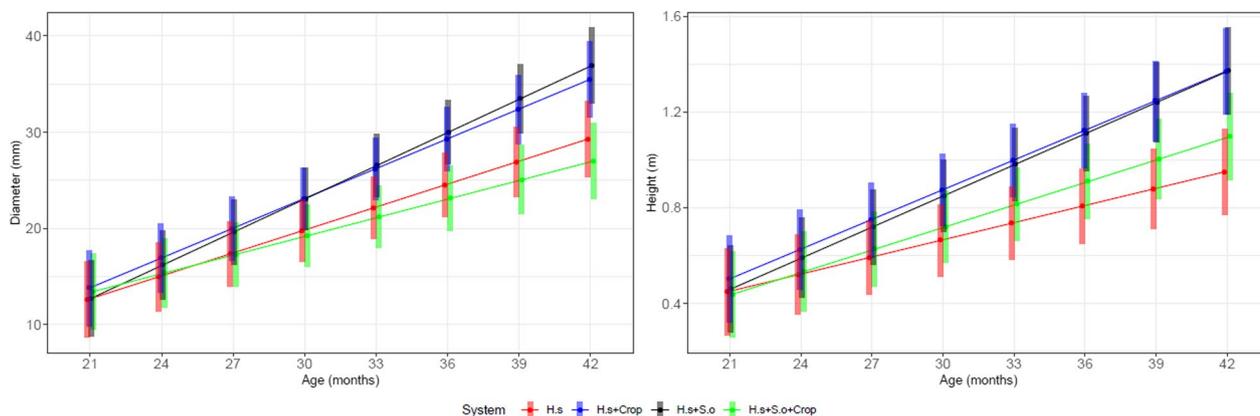


**Fig. 5** Effects of system and season on the pigment parameters ( $P < 0.05$ ). The blue bars are the confidence intervals of the means, and the red arrows are the comparisons between them. If an arrow from one mean overlaps the arrow of another treatment, there are no differences between means ( $P > 0.05$ ) by Tukey’s test. **Chl**—chlorophyll; **Flav** – flavonoids index; **Anth** – anthocyanins index; **NBI**—nitrogen balance (chlorophyll index/flavonoids index). **H.s. + S.o. + Crop**—*Hancornia speciosa* intercropped with annual crops (corn and pumpkin, in succession to each harvest sown between rows) and *S. oleracea* (‘Gueroba’) grown between the plants in the row. **H.s. + S.o.** – *Hancornia speciosa* intercropped with *Syagrus oleracea* (‘Gueroba’) cultivated between the plants’ rows. **H.s.**—*Hancornia speciosa* monoculture grown in full sun. **H.s. + Crop**—*Hancornia speciosa* intercropped with annual crops (corn and pumpkin, in succession to each harvest sown between rows)

The results show that double intercropping (annual crops or *S. oleracea*) provided cumulative increases in growth. In the triple intercropping system, there was no reduction in growth rate when compared to full sun. These findings show that designs that consider species, densities, and adequate times for establishment of intercropping systems provide more efficient, promoted

by positive interaction between species (Custódio et al. 2015; Gao et al. 2013; Santos et al. 2017a; Schwartz et al. 2015).

The triple intercropping has a spatial/temporal arrangement with plants arranged in line and between lines, surrounding the plants of *H. speciosa* on all sides (Fig. 3). In double intercropping, the arrangement is only



**Fig. 6** Effects of age on the stem diameter and height of *H. speciosa* ( $P < 0.05$ ). The bars represent the confidence intervals of the means. **H.s. + S.o. + Crop**—*Hancornia speciosa* intercropped with annual crops (corn and pumpkin, in succession to each harvest sown between rows) and *S. oleracea* ('Gueroba') grown between the plants in the row. **H.s. + S.o.**—*Hancornia speciosa* intercropped with *Syagrus oleracea* ('Gueroba') cultivated between the plants' rows. **H.s.**—*Hancornia speciosa* monoculture grown in full sun. **H.s. + Crop**—*Hancornia speciosa* intercropped with annual crops (corn and pumpkin, in succession to each harvest sown between rows)

on the line or between the lines, depending on the system tested. Competition for space, light, water and nutrients in the triple intercropping is naturally higher (Custódio et al. 2015; Gao et al. 2013; Santos et al. 2017a, b). The results presented demonstrate a tendency of etiolation in the triple intercropping, with inversely proportional height and diameter (Additional file 1: Figs. S9 and S10).

In the triple intercropping (H.s + S.o + Crop) the height of H.s was more favored than the stem diameter. In this aspect, it shows poor performance, since photosynthesis apparently has a more direct relationship with the growth in diameter than with the height of the plant (Lima et al. 2008). Now, in full sun (H.s) there was an investment opposite characteristics, with reduction of vegetative structures (Righi et al. 2016).

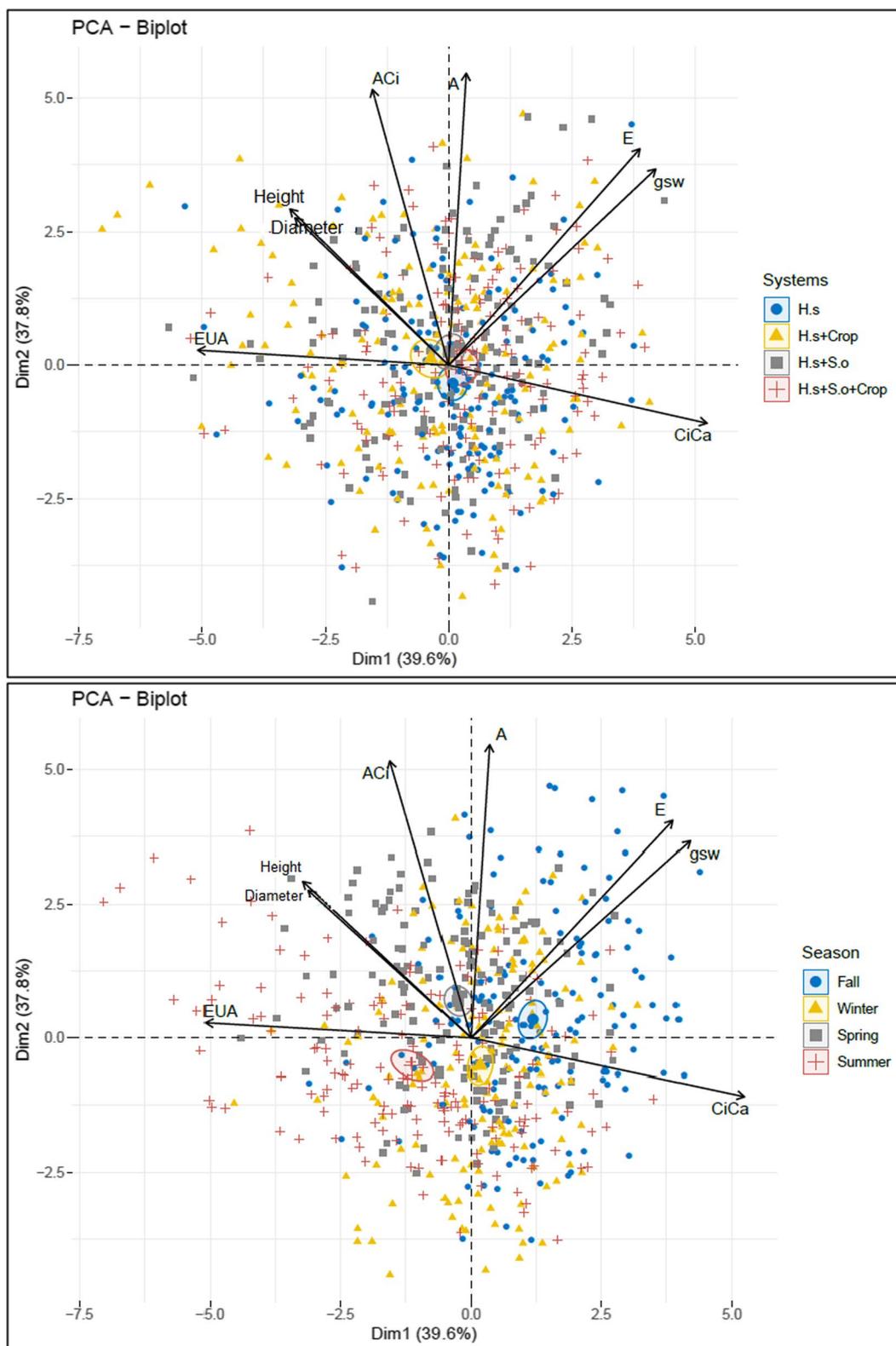
It should be noted that intense shading, as well as exposure to full sun, can cause stress due to lack or excess of light on plant leaves and consequently high leaf temperature (Taiz and Zeiger 2009). However, partial shading can contribute to the improvement of these microclimatic parameters. Plants have ideal ranges of leaf temperature and light intensity, which allow an increase in the CO<sub>2</sub> assimilation rate resulting from effects on carboxylation efficiency and stomatal conduction (Machado et al. 2005).

These results are directly related to the improvement and/or maintenance that the intercropping systems provided to the physiological vigor of *H. speciosa* plants throughout the seasons. In general, intercropped crops, especially double intercropping systems, provided higher photosynthetic rates and instantaneous carboxylation efficiency ( $A/C_i$ ), as shown in the PCA (Fig. 7A). Integrated systems promote a more favorable microclimate

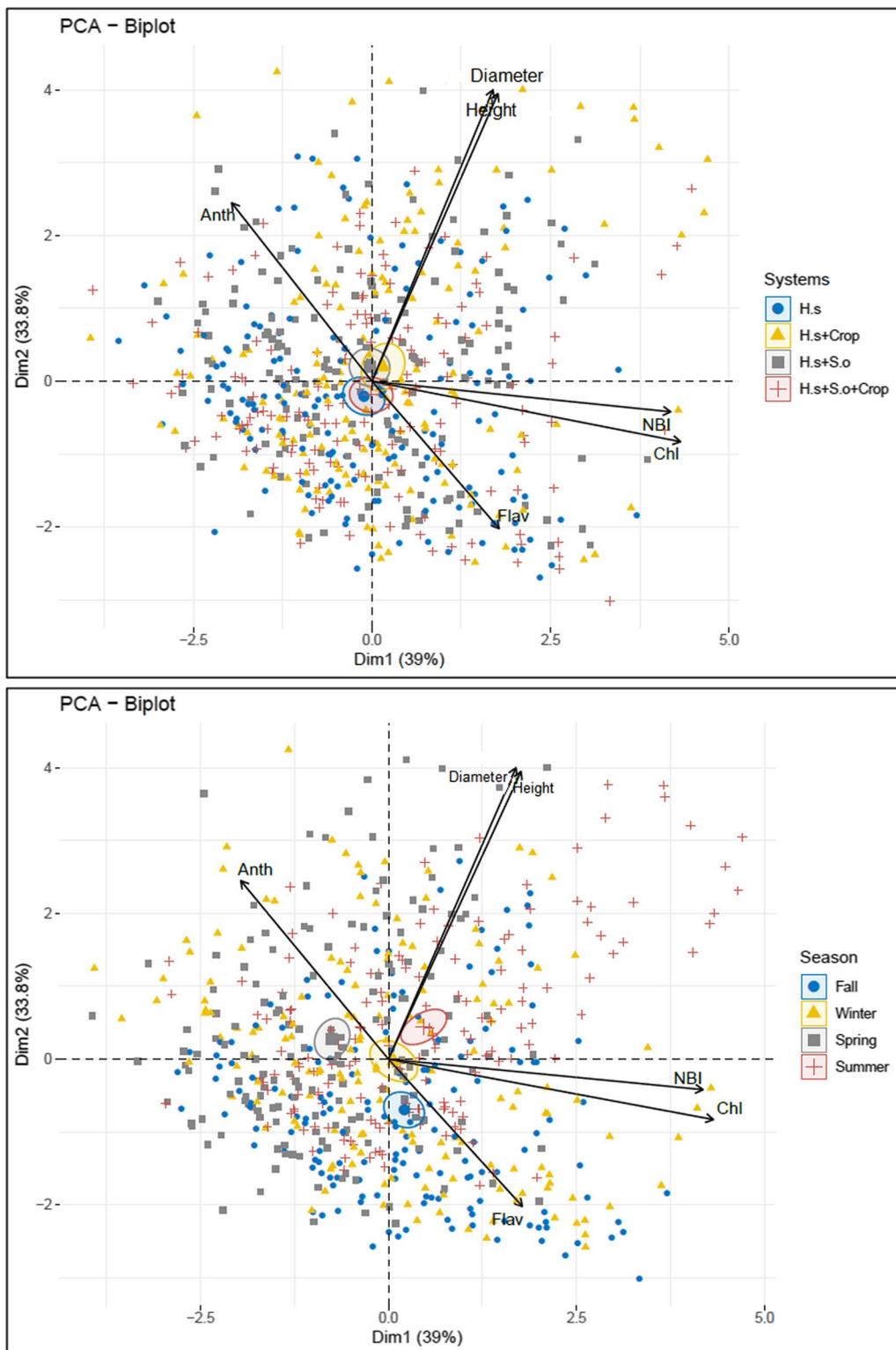
for crop development. Agroforestry systems act by changing the luminosity of the environment, decreasing the air temperature, especially under high temperature conditions. Additionally, humidity can be increased (Guo et al. 2017; Yang et al. 2021). This reduces the vapor pressure deficit (VPD), favoring stomatal opening and increasing gsw, optimizing carbon fixation, but without increasing the loss of water vapor in the leaves (Santos et al. 2017b). Since, adequate temperatures result in gains in the indecencies of gas exchange (Machado et al. 2005).

These responses were more evident in fall and winter, which are characterized by milder temperatures and low rainfall. Notably, these events were more pronounced in the winter, where there was an effective reduction in the photosynthetic rate in all cropping systems. However, over time the small physiological gains were linearly reflected in the accumulation of benefits from intercropping systems suitable for the establishment of *H. speciosa* orchards (Fig. 6).

The maintenance of the photosynthetic rate of these plants may be linked to the water relationships of the system. The plants grown in intercropping showed an overall increase in stomatal conductance. Especially in the fall when comparing H.s versus H.s+S.o and H.s+S.o+Crop. Therefore, they had a higher capacity to conduct higher carbon concentrations to the mesophyll, as indicated by  $C_i/C_a$ . This process improves the instantaneous carboxylation efficiency of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), since it concentrates more carbon near its carboxylic sites, increasing the probability of carboxylation occurring in relation to the oxygenation reactions that would lead the system to photorespiratory metabolism (Avila et al.



**Fig. 7** Multivariate analysis (PCA) and contributions of the gas exchange and biometric variables to the system (A) and season (B). Centroid and 95% CI for each season are shown. **A**—net carbon assimilation rate ( $\mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ); **E**—transpiration rate ( $\text{mmol H}_2\text{O}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ); **WUE**—water use efficiency ( $\mu\text{mol CO}_2/\text{mmol H}_2\text{O}$ ); **C<sub>i</sub>/C<sub>a</sub>**—internal CO<sub>2</sub> concentration/ambient CO<sub>2</sub> concentration ratio; **gsw**—stomatal conductance ( $\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ); **A/C<sub>i</sub>**—instantaneous carboxylation efficiency ( $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}/\mu\text{mol}\cdot\text{mol}^{-1}$ )



**Fig. 8** Multivariate analysis (PCA) and contributions of the pigment and biometric variables to the system (A) and season (B). Centroid and 95% CI for each season are showed. **Chl**—chlorophyll; **Flav** – flavonoids index; **Anth** – anthocyanins index; **NBI**—nitrogen balance (chlorophyll index/ flavonoids index)

2019). The lower PAR values found in triple intercropping associated with interspecies competition may have suppressed more expressive photosynthetic rates, as well as efficient water use (WUE) of *H. speciosa* plants.

The reduction in gas exchange rates observed between seasons was closely linked to the phenological stage and to stomatal limitations than photochemical limitation. In the spring, for example, there were reductions in the indicators of good functioning of the photochemical apparatus as evaluated based on the chlorophyll *a* fluorescence parameters, such as the potential quantum yield ( $F_v/F_m$ ) and the photosynthetic performance index (PIABS) (Gonçalves et al. 2010). In addition, there was an increase in  $F_o$  and energy dissipation in the form of heat, i.e., nonphotochemical quenching ( $\Phi_{DO}$  and  $DI_o/RC$ ). However, the photosynthetic rate (*A*) and instantaneous carboxylation efficiency of Rubisco ( $A/C_i$ ) had the highest mean values in spring comparing to other seasons.

Despite the correlation between PSII efficiency and net  $CO_2$  assimilation rate (Baker and Rosenqvist 2004), this study found that there may have been a discrepancy between the recovery of photochemical machinery damage still occurring in winter and improvement in the efficiency of gas exchange due to the reestablishment of the rainfall regime in mid-spring.

In contrast, in the summer, the resumption of PIABS and of chlorophyll concentration was observed, while there was a decline in gas exchange, which may be attributed to greater leaf expansion and high temperatures inherent to this season. A larger leaf area may have reduced chloroplast density in summer, and high temperatures suppress stomatal conductance, reducing the internal  $CO_2$  concentration and the transpiration rate, in contrast with high values of water use efficiency.

Furthermore, there is not always a positive correlation between photosynthetic rate measured by area and leaf

area, and in some cases even negative, so it is important to consider characteristics such as specific leaf area and leaf age (Bhagsari and Brown 1986; Weraduwege et al. 2015). Thus, the supply of photochemical products was sufficient to sustain the Calvin cycle, and the production of trioses that provided energy and carbon skeletons during plant growth was maintained (Guidi et al. 2019). Additionally, the possible explanation is that photochemical damage did not limit carboxylation.

In contrast, plants that are under any type of stress tend to reduce the transfer of photochemical energy and increase the rate of energy loss in the form of heat and fluorescence (Guidi et al. 2019).

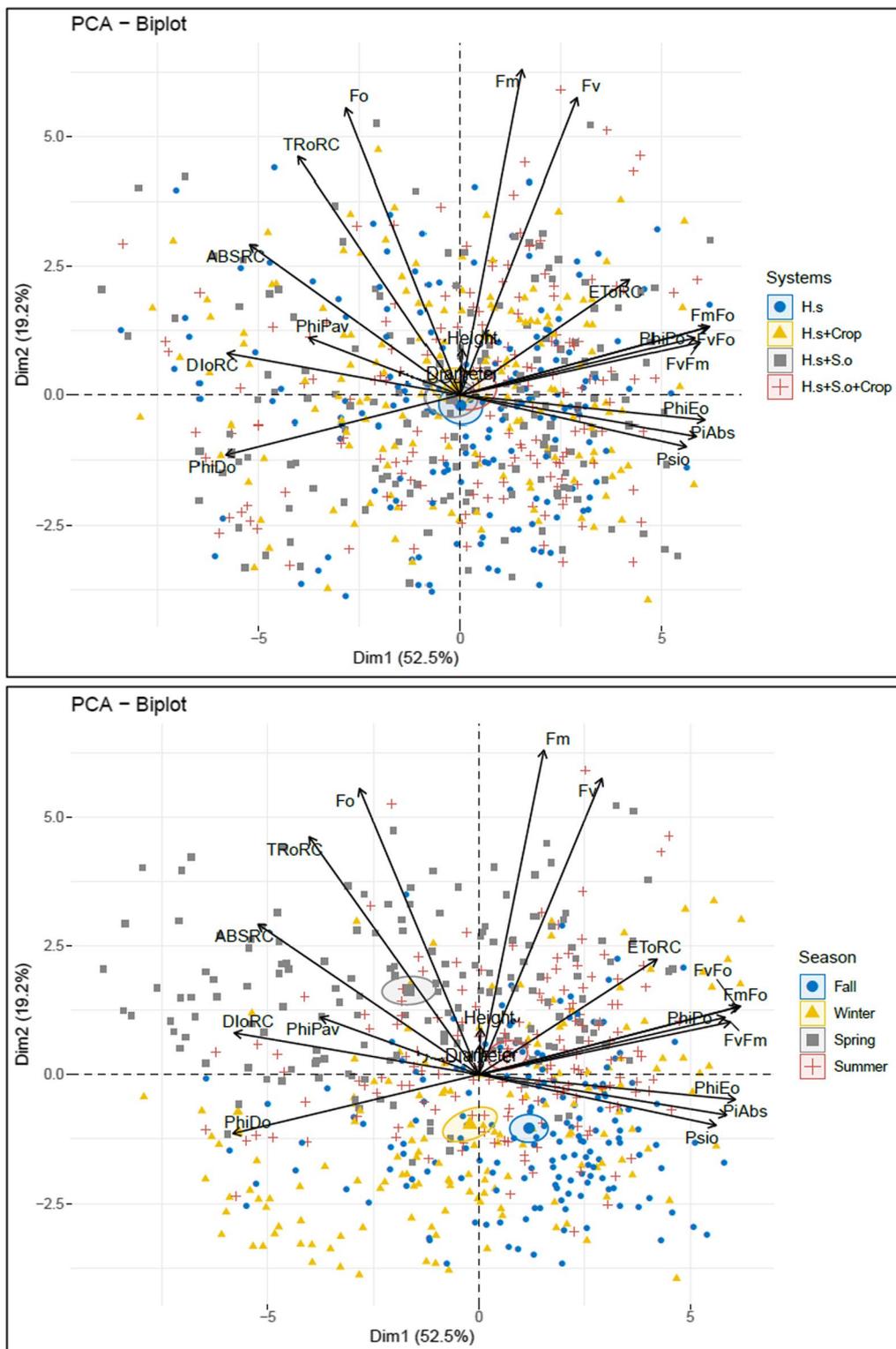
Therefore, our findings suggest that the senescence process initiated in the winter culminated in a reduction in the chlorophyll content in the spring, triggering a sequence of negative events on the photochemical stage. This result corroborates to the mechanisms described by Sakaigaichi et al. (2019), as they discovered that the chlorophyll content and the  $F_v/F_m$  ratio are common to decrease in winters in sugarcane crops.

It is important to highlight the literature lack of parameters related to chlorophyll *a* fluorescence in *H. speciosa*. Nevertheless, this native species, presented values known as “adequated” differing from the values found in crop species. For example,  $F_v/F_m$  values lower than 0.7 in sugarcane means photoinhibition (Sakaigaichi et al. 2019), however, in the present study we verified that  $F_v/F_m$  values up to 0.65 did not compromise the photosynthesis of *H. speciosa*. Further studies about chlorophyll *a* fluorescence in *H. speciosa* are essentials to improve knowledge about the physiology of this specie and expand their use.

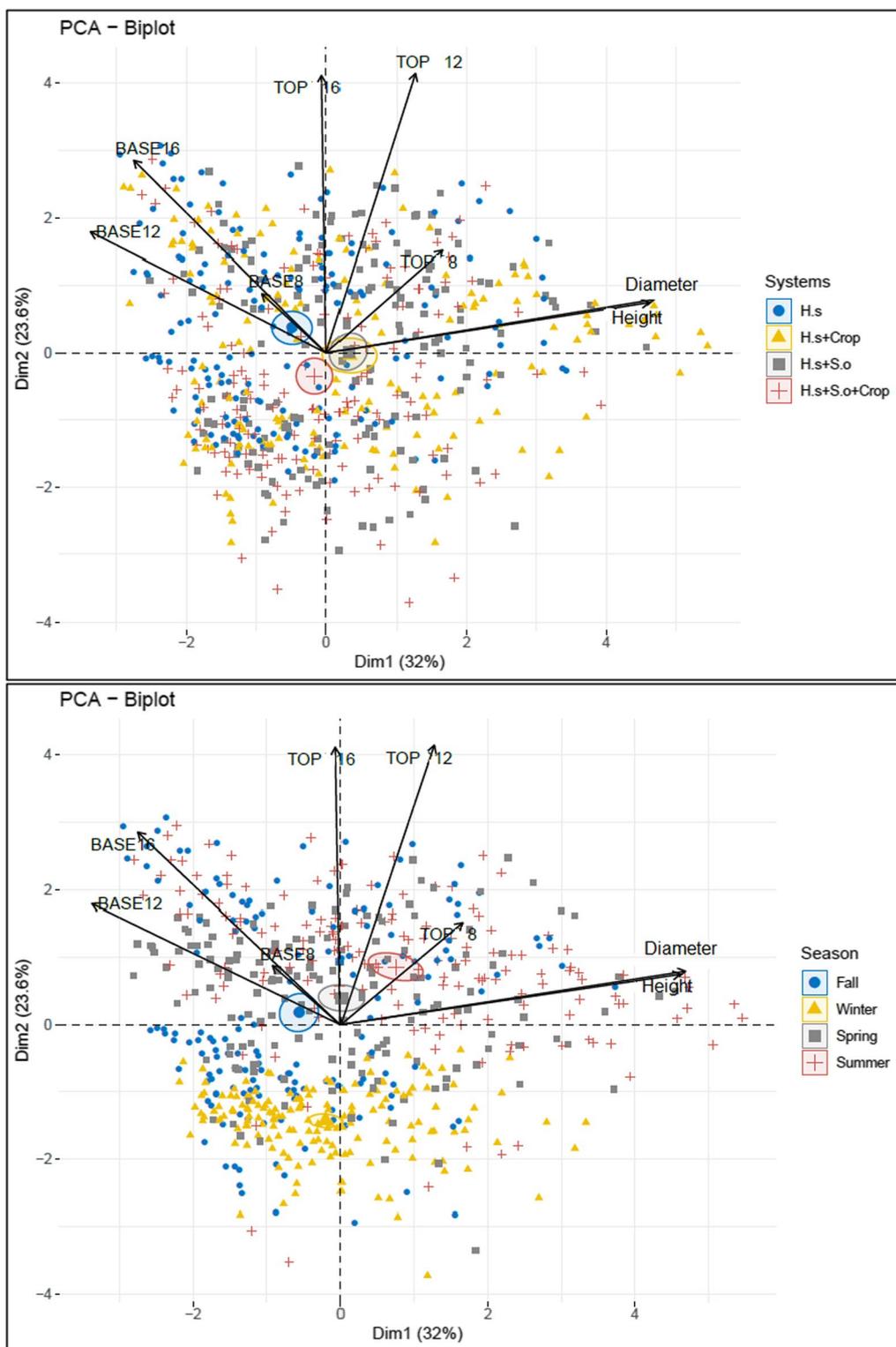
The possibility of classifying and/or describing a tree species through physiological attributes is already known (Bussotti and Pollastrini 2015; Pollastrini et al. 2016) and reinforced mainly when associated with multivariate

(See figure on next page.)

**Fig. 9** Multivariate analysis (PCA) and contributions of the fluorescence and biometric variables to the system (A) and season (B). Centroid and 95% CI for each season are showed. Initial fluorescence ( $F_o$  – electrons.quantum<sup>-1</sup>), represents all oxidized quinone A; Variable fluorescence ( $F_v$  – electrons.quantum<sup>-1</sup>) indicates damage to the antenna complex caused by stress; Maximum fluorescence ( $F_m$  – electrons.quantum<sup>-1</sup>) represents all reduced quinone, the higher the better the energy dissipation in the electron transport chain. The quantum efficiency index of FSII or potential photosynthetic activity (maximum quantum production ratio of competing photochemical and non-photochemical processes in FSII ( $F_v/F_o$  ou  $kP/KN$ —electrons quantum<sup>-1</sup>), demonstrates that this parameter is related to the energy captured and the energy dissipated. The index ( $F_m/F_o$ ) is the ratio between the maximum and initial fluorescence. PSII Quantum Efficiency, the lower the stress, the greater the stress, indicating damage to the photosynthetic apparatus ( $F_v/F_m$ ).  $\Phi_{P_o}$  or  $TR_o/ABS$  or  $\phi P_o$  is the maximum primary photochemical quantum yield index of PSII, non-photochemical dissipation of energy in the form of heat.  $\Phi_{E_o}$  or  $ET_o/ABS$  or  $\phi E_o$  or  $\Phi I(E_o)$  is the index that demonstrates the probability of an exciton moving an electron through the electron transport chain after Quinone A (Qa).  $\Psi_{t=0}$  is the probability, in time  $t=0$ , of a trapped exciton moving an electron through the electron transport chain after Qa-.  $\Psi_{ABS}$  or  $\psi_{ABS}$  is the performance index based on absorption, reflects the efficiency of light absorption between capture and transfer of FSII excitation, representing the dissipation of photochemical energy through the formation of ATP and NADPH.  $ABS/RC$  is the energy absorption per reaction center, the growth in this index indicates an apparent increase in the photosystem’s antenna complex to compensate for the high loss of energy as heat.  $\Phi_{D_o}$  or  $\phi D_o$  is the energy dissipation quantum yield.  $TR_o/RC$  is the flow of energy trapped by reaction center.  $ET_o/RC$  is the electron transport flow per reaction center.  $D_o/RC$  is the specific flow of energy dissipation per reaction center at the level of chlorophylls in the antenna complex, representing disorder at the beginning of the energy transport process.  $\Phi_{Pav}$  or  $\phi PAV$  is the time to reach maximum chlorophyll fluorescence (ms)



**Fig. 9** (See legend on previous page.)



**Fig. 10** Multivariate analysis (PCA) and contributions of the photosynthetically active radiation and biometric variables to the system (A) and season (B). Centroid and 95% CI for each season are showed. **BASE8**—Photosynthetically active radiation (PAR) was determine below the *H. speciosa* canopy at 8 am. **TOP8**—Photosynthetically active radiation (PAR) was determine above the *H. speciosa* canopy at 8 am. **BASE12**—Photosynthetically active radiation (PAR) was determine below the *H. speciosa* canopy at 12 pm. **TOP12**—Photosynthetically active radiation (PAR) was determine above the *H. speciosa* canopy at 12 pm. **BASE16**—Photosynthetically active radiation (PAR) was determine below the *H. speciosa* canopy at 4 pm. **TOP16**—Photosynthetically active radiation (PAR) was determine above the *H. speciosa* canopy at 4 pm

analysis techniques (Ganopoulos et al. 2015; Pollastrini et al. 2016), with the possibility of combined and automated use (Virlet et al. 2017) being a trend in production systems. Therefore, finding new production systems depends on the grouping of results, as it allows us to identify and group the different physiological patterns of *H. speciosa* under favorable and unfavorable conditions.

The gains in growth rate of *H. speciosa* obtained with the intercropping cultivation systems highlights the potential contribution of intercropping systems to food security and, at same time, to genetic resources and biodiversity conservation (Bhagwat et al. 2008; Bisseleua and Vidal 2008). It is noteworthy that the species used here are alternative to the portfolio of the main food species used in the world.

Further studies with this species is fundamental to determine the influence of the use of fertilizers between the rows of trees, as fertilizers were used for the nutrition of annual crops in the intercropping systems with annual crops (H.s + Crop and H.s + S.o + Crop). Indeed, long-term differentiation is expected, especially at the beginning of the reproductive phase with fruit production. This is because the intercropping system with S.o received fertilizers only in the palms located between *H. speciosa* plants.

## Conclusions

Overall performance of *H. speciosa* was favored by double intercropping; therefore, the best way to implement *H. speciosa* orchards is intercropped with annual crops or with *S. oleracea*. The physiological changes were subtle but detectable through the growth rate at 42 months after field establishment, demonstrating the need for long-term studies of this species.

Triple intercropping (H.s + Crop + So) did not negatively impair growth when compared to *H. speciosa* in full sun. Further studies should seek systems that decrease the effects of full sun, without promoting severe shading in the establishment of *H. speciosa*. It is noteworthy that the seasons of the year had a strong influence on the physiological and environmental parameters of *H. speciosa*.

## Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s43170-024-00235-0>.

**Additional file 1.** Effects of systems and seasons in pigments, fluorescence of chlorophyll, gas exchange, photosynthetically active radiation (PAR), and biometrics of *H. speciosa* plants.

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## Author contributions

EMA—Part of the author's doctoral thesis. Planning, conducting reviews and writing the article. FGS—Advisor of the author's doctoral thesis. Planning, conducting reviews and writing the article. RGA, LLL, TCdO, AMC, MR—Conducting, evaluating, and contributing to the writing of the article. JPP—Contributing to the writing of the article. TdoPP—Conducting, evaluating, statistical analysis and contributions in writing the article.

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## Availability of data and materials

Additional file 1 follows in the metadata.

## Declarations

### Ethics approval and consent to participate

The activities carried out in the construction of the article do not require evaluation by an ethics council.

### Consent for publication

The authors consent to CABI Agriculture and Bioscience to publish the full article.

### Competing interests

We declare that there is no conflict of interest in the realization of this paper.

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